Molecular profiling of selected rice landraces for drought stress tolerance and characterization of associated plant growth promoting rhizobacteria

Synopsis

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Rice is one of the principal staple crops of the world, especially in south-eastern Asia. It is the second highest producing crop of the world after corn providing one quarter of global per capita human energy. Rice is growing in a wide geographical distribution, spanning from 53° N to 45° S, even 3 m below sea level to as high as 3000 m. Being distributed throughout a wide geographical region across varied climatic conditions, rice has to face several biotic and abiotic stresses which are compounded by the abnormal behavior of global climatic conditions.

Among the different stresses drought stress is one of the most important limiting factors for rice growth and production. Drought can affect both the vegetative and reproductive stages of plants but in case of rice the effect of drought stress is more detrimental during the reproductive stage than vegetative stage posing a threat to rice production. Therefore, developing drought resistant lines and understanding the mechanisms of drought resistance are the utmost important for rice improvement as well as food security.

There are several strategies for drought management in rice like, selection of resistant lines, hybrid breeding, *in-vitro* selection of potent rice lines, trait linked marker assisted breeding, transgenic approaches, use of beneficial rice field microorganisms (PGPM) etc.

The main focus of the present study was exploration of valuable rice genetic resources and characterization of associated microbial resources in relation to drought stress tolerance of rice.

Researches have shown that rice landraces can provide a good source of diverse genes, which is very much essential for rice improvement programmes. For that reason some rice landraces grown in uplands of some drought prone districts of West Bengal were targeted for the study. To utilize these plant genetic resources proper and elaborate agro-morphological, physio-biochemical and molecular profiling were done.

As these landraces did not have any previous profiling report so some initial identification was done by documenting their panicle morphologies and grain-kernel dimensions. Based on the grain-kernel dimensions these rice landraces were categorized in terms of grain and kernel shapes. From the grain-kernel study it has

been found that the majority of the studied landraces have medium to bold grain and kernel morphology. Only Huggi Bhatta and Deula Bhog showed slender kernel shape.

In case of agro-morphological characterization 13 very commonly used traits (Grain length, Kernel length, Grain breadth, Kernel breadth, Grain shape, Kernel shape, 100 Grain weight, 100 Kernel weight, Plant height, Flag leaf length, Flag leaf breadth, Number of Panicle and Panicle length) were screened over the studied landraces.

Genetic stability (fixation of traits) is an important character for the selection of donor parent in any kind of breeding programmes, which was tested through heritability test. The heritability percentage of the studied landraces leveled to a high value, which indicates the stability of all the studied traits in the selected genotypes. From the high heritability values it can be assumed that the rest of the traits in the studied genotypes were also stable (fix). So, these selected landraces can be utilized as donor parent for breeding purposes.

For physiological screening two (germination percentage and seedling growth) important and most widely used characters were chosen. From the physiological screening it has been found that some of the studied rice landraces (BhutMoori, Kelas, Noichi, Kalodhan and Deula Bhog) performed better under drought stress conditions.

After physiological screening biochemical characterization was also done. For biochemical screening two common stress associated compounds (total chlorophyll and total protein) were targeted. From the total chlorophyll content study it has been found that among the studied landraces Noichi, Kelas and Bhut Moori showed higher chlorophyll content under different induced stress conditions whereas Huggi Bhatta showed lowest.

From the total protein profile it has been found that among the studied landraces Bhut Moori, Kalo Dhan, Rani Kajal and Deula Bhog have higher protein content under the stress conditions.

Three important oxidative stress tolerance linked enzymes (catalase, peroxidase and ascorbate peroxidase) were also assayed among the selected rice landraces for the studied conditions. From the catalase activity screening it has been found that all the studied genotypes have good level of catalase activity (more than 200 U/min/g F.W.) under control condition and catalase activity was increasing even

more under stress. Among the studied landraces Bhut Moori showed highest catalase activity under stress conditions. From the peroxidase assay it has been also found that Bhut Moori showed highest enzyme activity among the studied landraces, whereas Huggi Bhatta showed lowest activity under studied conditions. In case of ascorbate peroxidase assay Kelas showed highest activity whereas Bhut Moori showed second highest activity even at highest level of induced stress condition.

Assessment of osmotic adjustment capacity indicative of potential drought tolerance among the landraces showed that Bhut Moori has the highest level of proline, one of the most common osmolyte involved in osmotic adjustment against drought condition, whereas Tulsimukul has the lowest level under studied stress conditions.

For statistical validation of the generated data multivariate analysis was done, where it showed high degree of significance at $p<0.05$. Diversity was also analyzed for each data set yielded by each studied physio-biochemical parameter by Duncan's test, where all the data set showed high variability. Fisher's LSD analysis was also done for each data set to find out the better performers (landraces) under different induced stress conditions in comparison with two used checked varieties (IR64 and N22). From the LSD analysis (for each condition separately) it has been found that Bhut Moori showed highest degree of tolerance (in terms of higher values of the selected parameters). It has been also found that majority of the studied rice landraces (like Bhut Moori, Noichi, Huggi Bhatta, Kalodhan, Deula Bhog and Rani Kajal) have performed better than the drought tolerant checked variety (N22) under given stress conditions. So, some of the above mentioned varieties (especially Bhut Moori) showed high potentiality related to drought stress tolerance.

For molecular profiling genotyping was done based on some osmotic stress tolerance linked SSR and ISSR markers. The fingerprint profile showed high degree of allelic variation among the studied rice genotypes, indicating the rich source of genetic diversity which is very much essential for marker assisted breeding. The allelic diversities were presented in form of some panels. The panel is self explanatory to highlight different information such as, markers used, genotypes used, all the alleles generated, molecular weight of all the alleles etc. From the panel lot of other

valuable information (number of alleles, allelic frequency, polymorphic nature of a used marker or genotype etc) can also be generated.

Fingerprint analysis showed that RM107 has the highest number (6) of alleles among the studied SSR markers. On the other hand, RM162 showed highest allelic difference (114.25 bp) and RM10764 showed lowest (6.45 bp). The total numbers of alleles found in case of studied polymorphic SSRs are 98. In case of ISSR markers PR36 showed highest number of allele (9) among the used ISSR markers whereas PR39 showed highest (635.07 bp) allelic difference and PR29 showed lowest (15.36) difference. The total numbers of alleles found in case of ISSR markers are 52. Some unique alleles were also found in case of both the marker types, which can be utilized for varietal identification as well as IPR (Intellectual Property Rights) related issues.

To find out the informativeness of the used markers Polymorphism Information Content (PIC) was calculated, where it has been found that in case of SSR markers RM10793 showed highest polymorphism (PIC= 0.7927) whereas RM136, RM10748 and RM10764 showed lowest (0.3548). On the other hand, in case of ISSR markers PR36 showed highest informativeness (0.8633) whereas PR39 showed lowest (0.5442). So, in total some of the used markers (like RM107, RM242, RM8094, RM10720, RM10793, RM10890, PR31, PR34, PR36, PR40 and PR41) showed high amount (more than 0.7000 of PIC value) of informativeness, which can be utilized for future purposes.

For the diversity analysis dendrograms were constructed for the studied two types of markers. The dendrogram generated from SSR markers have two major clusters (Bhut Moori- N22- Kelas- Kalodhan- Noichi- Baid Dhusuri- Deula Bhog- Gorah- RaniKajal in one cluster and Tulsimukul- IR64- Kalo Nuniya- Huggi Bhatta in another cluster). In the first major cluster Bhut Moori- N22 and Deula Bhog- Gorah are closely associated with each other. On the other hand, Tulsimukul and IR64 are close to each other in second major cluster. From the dendrogram generated from ISSR markers it was found that it also have two major clusters where, Bhut Moori- N22- Kalodhan- Noichi- Kelas- Rani Kajal- Deula Bhog- Gorah form one cluster and Huggi Bhatta- IR64- Tulsimukul- Baid Dhusuri- Kalo Nuniya form the another cluster. In the first major cluster Bhut Moori- N22 and Rani Kajal- Deula Bhog are closely associated with each other. On the other hand, Huggi Bhatta and IR64 are

closely associated with each other in second major cluster. From the cluster analysis it has been found that all the better performed varieties (in the studied stress conditions for the physio-biochemical screening) are clustered with the drought tolerant checked variety (N22) whereas the less performed varieties (like Tuksimukul, Huggi Bhatta etc) clustered with the drought susceptible checked varietty (IR64). So, the physio-biochemical screening was also verified by genotyping study.

Based on the physiological, biochemical and molecular screening Bhut Moori has been selected for expression study. The drought tolerant property of Bhut Moori was checked in a more precious manner by expression analysis (through qPCR). For qPCR experiments two important markers (*p5cs* and *catA*) associated with drought stress were utilized. From the expression analysis it has been found that both the genes were upregulated much higher in case of Bhut Moori than the reference genotype (N22) under control as well as induced stress conditions.

So, based on the present study Bhut Moori may be recommended for utilization as donor parent for drought stress tolerance linked rice improvement programmes.

For utilization of microbial resources in relation to drought stress some rice rhizospheric soil samples were collected to isolate some potential candidates [rice rhizospheric PGPR (plant growth promoting rhizobacteria)]. From the collected soil samples 16 different isolates were isolated through pure culture technique. The isolates were screened for the PGPR activities through phosphate solubilization, indole acetic acid (IAA) production, Nitrogen fixatation and ACC Deaminase production. Based on the PGPR activity screening, isolate 1 (I1), isolate 2 (I2) and isolate 6 (I6) have been selected for further study.

Effect of the selected isolates on plant growth under the studied drought stress condition (-0.5 MPa, -1.0 MPa and -1.4 MPa) was determined for the drought susceptible variety IR64. From the screening it has been found that both the I1 and I6 have positive effect on plant growth and development under control as well as different given drought stress conditions.

For the identification of these two potential candidates some phenotypic as well as genotypic (16S rDNA sequencing) characterization was done. From the

identification it has been found that the isolate 1 is a *Mycobacterium* sp and the isolate 6 is a *Bacillus* sp. This may be the first report from India where *Mycobacterium* strain have positive role in drought stress tolerance of rice.

So, from this study it can be concluded that these two isolates have potentiality to help rice plants under drought stress conditions.

Finally it can be concluded that as a source of rice genetic resource Bhut Moori may be recommended for donor parent in drought stress tolerance linked rice improvement programmes, which need to be verified under field conditions also. On the other hand, two rhizospheric bacterial strains (I1 and I6) have been isolated for management of drought stress in rice that required further study (under field conditions) before appication in agriculture.

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